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12 PERSONAL AUTHOR(S)

Sheldon Cohen, Carnegie Mellon University & David Tyrrell, Common Cold Unit Medical Research Council

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Our study involves predicting infection and symptomatology in humans from psychological and immunologic measures taken prior to a viral challenge (Rhinoviruses 2, or 9, or 14, or Corrona virus 221E). Subjects are approximately 1,000 volunteers kept in isolation and monitored closely for viral shedding, and symptomatology over nine-day trails. ONR funds are primarily used to examine the usefulness of antibody titers to herpes simplex virus 1 (HSV-1) as an indirect measure of cellular immune function in this context. At study completion, HSV-1 antibody titers will be correlated with psychological measures, other humoral and cellular measures of immunity, and will be tested as a possible predictor of susceptibility to infection and clinical disease among infected subjects. The first year of funding has been spent hiring and training technicians and developing HSV-1 assays. An ELISA assay for IgG has been developed for the whole viral antigen and an IgA assay is also in development with the current

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19. ABSTRACT

proposed protocol involving an indirect ELISA and test-sera pre-absorbed with protein A. Measurement of specific IgM was considered but a preliminary trial revealed negligible amounts in sera with high IgG and IgA titers. Necessary antigens are being obtained for development of an anti-rubella antibody assay to provide a control for humoral (as opposed to cellular) implications of HSV-1 activation. Keywords:

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ANNUAL REPORT

Office of Naval Research Contract
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January 1, 1988 - December 31, 1988

The purpose of the research is to determine the roles of stress and natural social support systems in individual susceptibility to upper respiratory infection, and clinical colds. We also focus on the role of immunologic function in predicting susceptibility to colds and in linking stress and support to susceptibility. The general design involves predicting infection and symptomatology in humans from psychological and immunologic measures taken prior to inoculation with a cold virus (Rhinoviruses 2, or 9, or 14, or Corrona virus 221E). Subjects are approximately 1,000 volunteers kept in isolation and monitored closely for viral shedding, and symptomatology over nine-day trails.

ONR funds are used to examine the usefulness of antibody titers to herpes simplex virus 1 (HSV-1) as an indirect measure of cellular immune function in this context. This work was stimulated by Glaser's argument (e.g., Glaser & Gotlieb-Stematsky, 1982) that latent herpes viruses are held in check by cellular immune function and hence virus activation among HSV-1 infected persons suggests a suppression of cellular immune competence. In our study, HSV-1 activation is (a) correlated with various psychological measure; (b) compared with viral specific and nonspecific antibody titers, and with white blood cell counts including lymphocyte subset; and (c) evaluated as a predictor of susceptibility to infection and clinical disease. During the first year of the project we hired and trained technicians and developed appropriate assays for detection of HSV-1 antibodies. Data collection will be completed by June 1989 and all of the HSV-1 assays will be conducted at that time.

A review of the literature concerning human antibody response to HSV-1 revealed that, while some antibody rises were found during recurrence, the majority of investigations had failed to detect rises corresponding to virus reactivation. Although an assay of immunoglobulin G (IgG) against whole virus antigen is necessary to compare with the work of Glaser and his colleagues, it was considered that assay of IgA levels and IgG against more specific viral antigens would possibly be better indicators of viral reactivation. Enzyme labelled immunosorbent assays (ELISAs) were selected as being the most suitable and sensitive for the task.

To assay immunoglobulin against whole virus antigen an accredited HSV-1 strain has been obtained (strain 10798 from the Centre for Applied Microbiological Research) and a pool of viral antigen has been prepared after growing the virus in human diploid fibroblasts.

A control antigen pool has been prepared from uninfected cell cultures. A pool of IgG-positive sera has also been selected, for use as a positive standard in the IgG assays. The format for the IgG ELISAs is similar to the protocol we employ for antirhinovirus antibody assays. One half of the microtitre plates are coated with viral antigen and the other half with control antigen. A titration of the positive serum standard and duplicate samples of each test serum (at a single dilution) are added to each half of the plate. After reading the final optical densities from the assays, the control antigen values are subtracted from the viral antigen values. The positive serum standard is assigned an arbitrary value of 1×10^5 units so the corrected optical densities from the test samples can be equated with a point on the standard dilution curve and allocated a value. We will attempt to calibrate the positive serum standards against commercial sera of known immunoglobulin content, thus enabling the specific antibody content of the test-sera to be expressed in ng ml^{-1}. Test-sera are not given a value if their optical densities fall outside the calibration curve and the sera are retested at a different dilution. Sera are also retested if there is a large variation between the duplicate samples.

In pursuit of a more specific assay, monoclonal antibodies were obtained against viral glycoproteins D and B. In preliminary trials, viral antigen, which had been detergent solubilised, was captured by the monoclonal antibodies and this was recognised by human IgG. It could not be proven, however, that the monoclonal antibodies catured purified glycoprotein and discussion with other virologists indicated that glycoproteins tend to form heterologous aggregates and that it might be impossible to capture pure glycoprotein in its native form.

As an alternative means of measuring antibody against a specific glycoprotein, samples of glycoprotein D expressed in a baculovirus (grown in an insect cell line) were obtained. This proved successful as an antigen for ELISAs but assaying a selection of human sera revealed that the antibody activities detected were very similar to those obtained with the assay using whole viral antigen.

It is possible that antibody raised against viral early antigens/control proteins are less persistent than those against glycoprotein and are, therefore, better indicators of viral reactivation. Some of these early antigens have been successfully expressed in cell culture systems and contact has been made with someone working in this field with a view to eventually obtaining some antigen for use in assays.

It was hoped to measure anti HSV-1 IgA using a simple "Indirect" assay (as for IgG) but pre-absorption of the test-sera with protein-A (to remove IgG) resulted in higher IgA measurements. This indicated that the presence of IgG in unabsorbed sera was artificially depressing the perceived IgA titers presumably due to competition for antigen binding sites. The most convenient form of ELISA to overcome this problem would be an IgA capture assay but despite trials with different reagents and protocols the background optical densities

with capture assays could not be reduced to an acceptable level. This appeared to be due to non-specific absorption of the viral antigen. In view of this impasse, the protocol now adopted for IgA measurement utilizes an indirect ELISA and test-sera pre-absorbed with protein-A. A suitable pool of IgA-positive sera has been selected for use as a standard, so assay of the test-sera can now be begun.

Measurement of specific IgM was considered but a preliminary trial revealed negligable amounts in sera with high IgG and IgA titres. Other workers have reported measurable quantities of specific IgM only in response to primary or severe recurrent infections.

Before deciding upon the desirability of assaying the convalescent sera (returned by volunteers after departure from the Common Cold Unit) it was decided to test both pre infection and convalescent sera from the first 100 volunteers and review the results. This has now been completed for IgG and the IgA assays are about to be started. The results should also indicate whether it is necessary to look for IgA in sera which contain no anti HSV-1 IgG.

Because increased antibody production to HSV-1 could reflect a general activation of humoral immunity as opposed to cellular immune suppression, we decided to use anti-rubella antibody in test sera to control for this alterantive interpretation. An approach has been made to the Central Public Health Laboratory regarding their protocol for such assays and the purchase of necessary antigens; a reply is being awaited.

References

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Behavioral Immunology Program

Annual, Final and Technical Reports (one copy each except as noted)

INVESTIGATORS

Dr. Itamar B. Abrass
Department of Medicine
University of Washington
Harborview Medical Center
Seattle, WA 98104

Dr. Prince K. Arora
NIDDK, Bldg. 8, Rm. 111
National Institutes of Health
Bethesda, MD 20892

Dr. Andrew S. Baum
Department of Medical Psychology
Uniformed Services University
of Health Sciences, B3050
4301 Jones Bridge Road
Bethesda, MD 20814-4799

Dr. Charles A. Bowles
Merrifield Research Lab, Inc.
P.O. Box 2362
Merrifield, VA 22116-2362

Dr. Karen Bulloch
Helicon Foundation
4622 Sante Fe Street
San Diego, CA 92109

Dr. Michael D. Cahalan
Department of Physiology and Biophysics
University of California, Irving
Irvine, CA 92717

Dr. Donald A. Chambers
Health Sciences Center
University of Illinois at Chicago
P.O. Box 6998
Chicago, IL 60680

Dr. Christopher L. Coe
Department of Psychology
Harlow Primate Laboratory
University of Wisconsin
Madison, IL 53715

Dr. Sheldon Cohen
Department of Psychology
Carnegie-Mellon University
Pittsburgh, PA 15213

Dr. Walla L. Dempsey
Dept. of Microbiology and
Immunology
The Medical College of
Pennsylvania
3300 Henry Avenue
Philadelphia, PA 19129

Dr. David L. Felten
Department of Anatomy
University of Rochester
School of Medicine
601 Elmwood Avenue
Rochester, NY 14642

Dr. John F. Hansbrough
Department of Surgery
UCSD Medical Center
225 Dickinson Street
San Diego, CA 92103

Dr. Robert L. Hunter
Department of Pathology
Emory Univ. School of
Medicine
WMB 760
Atlanta, GA 30322

Dr. Terry C. Johnson
Division of Biology
Ackert Hall
Kansas State University
Manhattan, KS 66506

Dr. Sandra Levy
University of Pittsburgh
School of Medicine
3811 O'Hara Street
Pittsburgh, PA 15213

Dr. Lester Luborsky
Department of Psychiatry
308 Piersol Building/G1
University of Pennsylvania Hospital
Philadelphia, PA 19104

Dr. Steven F. Maier
Department of Psychology
University of Colorado
Campus Box 345
Boulder, CO 80309

Dr. Diana S. Malcolm
Department of Surgery, USUHS
Uniformed Services University
of Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20814-4799

Dr. Michael H. Melner
Department of Reproductive Biology
Oregon Regional Primate Center
505 N.W. 185th Avenue
Beaverton, OR 97006

Dr. Vera B. Morhenn
Department of Dermatology
Stanford University Medical School
Stanford, CA 94305

Dr. Jose R. Perez-Polo
Gail Borden Bldg., Rm. 436
University of Texas Medical Branch
Galveston, TX 77550-2777

Dr. Howard R. Petty
Department of Biological Sciences
Wayne State University
Detroit, MI 48202

Dr. Bruce S. Rabin
Clinical Immunopathology
Childrens Hospital
University of Pittsburgh School of
Medicine
Pittsburgh, PA 15213

Dr. Seymour Reichlin
Director, Clinical Study Unit
New England Medical Center Hospitals, Inc.
171 Harrison Avenue
Boston, MA 02111

Dr. Eric M. Smith
Department of Psychiatry
University of Texas
Medical Branch
Galveston, TX 77550

Dr. Ross R. Vickers, Jr.
Naval Health Research Ctr.
Bldg. 346
P.O. Box 85122
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ADMINISTRATORS

Dr. Jeannine A. Majde, Code 1141SB (2 copies)
Scientific Officer, Immunology Program
Office of Naval Research
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